(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 August 2000 (24.08.2000)

PCT

(10) International Publication Number
WO 00/49410 A3

(51) International Patent Classification[7]: G01N 33/68, 1/30, B01L 3/00

(21) International Application Number: PCT/US00/04023

(22) International Filing Date: 16 February 2000 (16.02.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/120,288 16 February 1999 (16.02.1999) US

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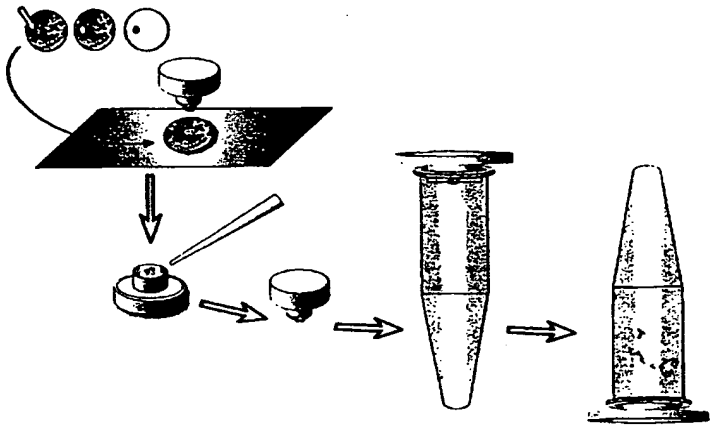
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: LCM (LASER CAPTURE MICRODISSECTION) FOR CELLULAR PROTEIN ANALYSIS



(57) **Abstract:** The present invention describes devices and methods for performing protein analysis on laser capture microdissected cells, which permits proteomic analysis on cells of different populations. Particular disclosed examples are analysis of normal versus malignant cells, or a comparison of differential protein expression in cells that are progressing from normal to malignant. The protein content of the microdissected cells may be analyzed using techniques such as immunoassays, 1D and 2D gel electrophoresis characterization, Western blotting, liquid chromatography quadrapole ion trap electrospray (LCQ-MS), Matrix Assisted Laser Desorption Ionization/Time of Flight (MALDI/TOF), and Surface Enhanced Laser Desorption Ionization Spectroscopy (SELDI). In addition to permitting direct comparison of qualitative and quantitative protein content of tumor cells and normal cells from the same tissue sample, the methods also allow for investigation of protein characteristics of tumor cells, such as binding ability and amino acid sequence, and differential expression of proteins in particular cell populations in response to drug treatment. The present methods also provide, through the use of protein fingerprinting, a rapid and reliable way to identify the source tissue of a tumor metastasis.

WO 00/49410 A3

Published:
— *With international search report.*

(88) Date of publication of the international search report:
8 March 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 00/04023

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N1/30 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMONE N L ET AL: "Laser-capture microdissection: opening the microscopic frontier to molecular analysis" TRENDS IN GENETICS,NL,ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 14, no. 7, 1 July 1998 (1998-07-01), pages 272-276, XP004124689 ISSN: 0168-9525 figures 2,3 ---	1,8-10, 12,13, 15,17, 20-24
X	US 5 843 657 A (BUCK MICHAEL E ET AL) 1 December 1998 (1998-12-01) cited in the application examples 4,5 column 7, line 51 - line 54 --- -/--	1-5, 8-13,15, 17-24, 35-41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2000	29. 11. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 00/04023

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	BANKS ROSAMONDE E; DUNN MICHAEL J ET AL.: "The potential use of laser capture microdissection to selectively obtain distinct populations of cells for proteomic analysis - Preliminary findings" ELECTROPHORESIS, vol. 20, April 1999 (1999-04) - May 1999 (1999-05), pages 689-700, XP000925546 the whole document ---	1-5, 8-13,15, 17-24, 35-41
P,X	SIMONE N L; REMALEY A T; PETRICOIN E F; GLICKMAN J W; BONNER R; EMMERT-BUCK M R; FLEISCHER T A; LIOTTA L A: "PSA quantitation in prostate cancer tissue cells procured by laser capture microdissection" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING (90TH ANNUAL MEETING, PHILADELPHIA, PENNSYLVANIA, USA; APRIL 10-14, 1999), vol. 40, March 1999 (1999-03), page 411 XP000929252 abstract ---	13,14
P,X	EMMERT-BUCK M R; ORNSTEIN D K; GILLESPIE J W; PAWELETZ C P; VOCKE C D; ET AL.: "Protein fingerprinting of LCM-dissected human esophageal and prostate cancer by 2D-PAGE" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING (90TH ANNUAL MEETING, PHILADELPHIA, PENNSYLVANIA, USA; APRIL 10-14, 1999), vol. 40, no. 526, March 1999 (1999-03), page 526 XP000929253 abstract ---	13,14
T	EMMERT-BUCK MICHAEL R; GILLESPIE JOHN W ET AL.: "An approach to proteomic analysis of human tumors" MOLECULAR CARCINOGENESIS, vol. 27, March 2000 (2000-03), pages 158-165, XP000925515 the whole document --- -/--	1-29, 35-41

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 00/04023

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	CAZARES L H; GONG L; NASIM S; SCHELLMAMMER P F; WRIGHT G L JR: "Discovery of prostate cancer biomarkers from laser capture microdissected (LCM) cells using innovative ProteinChipTM SELDI mass spectroscopy" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING (91ST ANNUAL MEETING, SAN FRANCISCO, CALIFORNIA, USA; APRIL 01-05, 2000), March 2000 (2000-03), page 851 XP000929268 abstract --- US 4 976 957 A (BOGOCH SAMUEL) 11 December 1990 (1990-12-11) examples 1-5 --- DATABASE WPI Week 198703 Derwent Publications Ltd., London, GB; AN 1987-017788 XP002143852 NOGUCHI JUJI ET AL.: "Protein from animal cerebral nerve tumour cell ..." & JP 61 275221 A (MITSUI PHARMACEUT. INC.) , 5 December 1986 (1986-12-05) abstract -----	13,14
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/US 00/04023

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-29, 35-41

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-29,35-41

   Methods of analysing proteins or other cellular components involving the extraction of a population of cells from a tissue sample using laser capture microdissection.

2. Claims: 30-34

   Devices comprising chambers having at least one input port.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/US 00/04023

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